Investigation of Low Temperature Plasma Capabilities to Modify the Structure and Function of Bio-Polymers

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Abstract:

The possibility to modify biopolymers using low temperature plasma is investigated. Two types of plasma are employed in this study: an atmospheric pressure plasma and a low pressure microwave plasma. In both cases changes of the structure and function of the exposed molecules are reported, the effects being stronger for the low-pressure plasma irradiation.

Keywords: *biopolymers, biomolecules, low temperature plasma, modification, plasma processing.*

1. Introduction

Applications involving low temperature plasmas have been developed in the recent years. All of them take advantage of the cold plasmas features of being suitable for heat resistant materials treatment, being non-toxic, a better alternative to chemical modification, and many others. Low-pressure plasmas, but also atmospheric pressure plasmas, have been employed in such applications [1]-[4]. The aforementioned have even more advantages, since they don't need a vacuum system and have a simpler electrode configuration, being easier to manipulate.

Biopolymers are polymers produced in the living organisms and involved in biological processes. Well known molecules among them are DNA, enzymes, starch. We are interested in proteins, which are biopolymers containing amino acids as small repetitive structure. Proteins are very common in living organisms from bacteria spores to human body, participating in almost every process in the body, and having various functions from enzymatic to forming the cytoskeleton of cells. The primary structure is chain of amino-acids linked by peptide bonds, the secondary structure is formed by regularly repeating local structures, involving hydrogen bonds, while the tertiary structure consists of several secondary structures stabilized by non-local interactions as hydrophobic, salt bridges, hydrogen bonds, disulfide bonds, etc. The organization process for the tertiary structure is called folding. Some proteins have even a quaternary structure where some sub-protein subunits bind together. The four levels of spatial organization make proteins bio-molecules with a complicated three-dimensional structure. Although the atomic species present in proteins are limited, and there are only 20 essential amino – acids involved in protein structure, the numerous type of bonds and number of amino – acid residues make protein molecules very complex and very stable.

2. Material and Method

Low temperature plasma processing of proteins would take advantage of all the features offered by these plasmas. In order to elucidate the mechanisms involved in plasma – protein interaction, we focused on simpler molecules due to the complicated structure of proteins, which makes it almost impossible to figure out the modifications that occurred under plasma exposure [5], [6].

Our study involved amino acids and peptides. A peptide is a bio-molecule similar to protein, made of up to 50 amino acids chained by peptide bonds.

As bio-molecules used to test low temperature plasma treatment potency, we have chosen a nonapeptide, Arginine Vasotocin (AVT or [Arg^8] Vasotocin). [Arg^8] Vasotocin is a peptide Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly-NH_2 with a disulfide bond between Cys^1 - Cys^6, having the chemical structure $C_{43}H_{67}N_{15}O_{12}S_2$. AVT is the progenitor of all vertebrate neurohypophyseal hormones. Among other functions, Arginine Vasotocin is involved in the osmoregulation process in some non-mammals [7], [8], [9]. The samples were prepared by solving AVT amorphous powder in pure water, placing the solution on small Silicon plates and drying them, all of these in order to obtain a homogenous treatment of the sample.

Atmospheric pressure plasma and microwave excited surface wave low-pressure plasma were used to investigate the effect of cold plasmas on these bio-molecules in different conditions. In both cases plasma was optically and electrically diagnosed. For the atmospheric pressure plasma we considered two different regimes of the discharge due to the nature substrate sample: conductive (silicon) or dielectric (glass). The modifications produced on the bio-molecule by both types of discharges were tested by means of presence of biological function, structure modification investigated by X-Ray Photoelectron Spectroscopy (XPS), and Time of Flight Mass Spectrometry (TOF-MS).

The configuration used to produce the atmospheric pressure plasma, presented in Figure 1, is very simple: a high voltage pulse is applied on an electrode placed around a glass tube. The discharge gas in our experiments was Helium.

The low-pressure plasma was produced in a stainless steel chamber of 17 cm height and 25.5 cm diameter using Argon as discharge gas and 500 W microwave power [10]. The samples were placed at about 8 cm inside the chamber. For all the experimental conditions the treatment time was 10, 20, 30 minutes, respectively. In each case control samples were prepared for each set of exposed items.

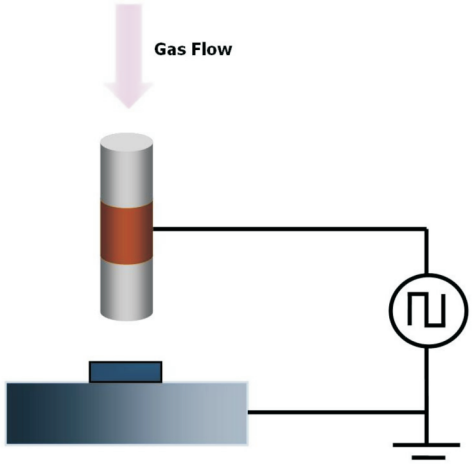

Fig. 1. Atmospheric pressure plasma device.

3. Results and discussions

3.1. Atmospheric pressure plasma treatment

We investigated the optical emission spectra for both regimes produced in the case of atmospheric discharge and we concluded that for the silicon substrate, the relative intensity of the light emitted by the plasma is almost twice stronger than for the glass substrate. Moreover, in the former case the emission is recorded due to more species, compared to the latter case (mainly radicals containing oxygen). These findings made us decide that the discharge produced when the irradiated substance is placed on conductive sample is more suitable for bio-molecule structure modification.

Vasotocin samples (on silicon substrate) were treated at an applied voltage of 10 kV peak to peak for 10, 20 and 30 minutes. The XPS spectra of control and 30 minutes samples are presented in Figure 2.

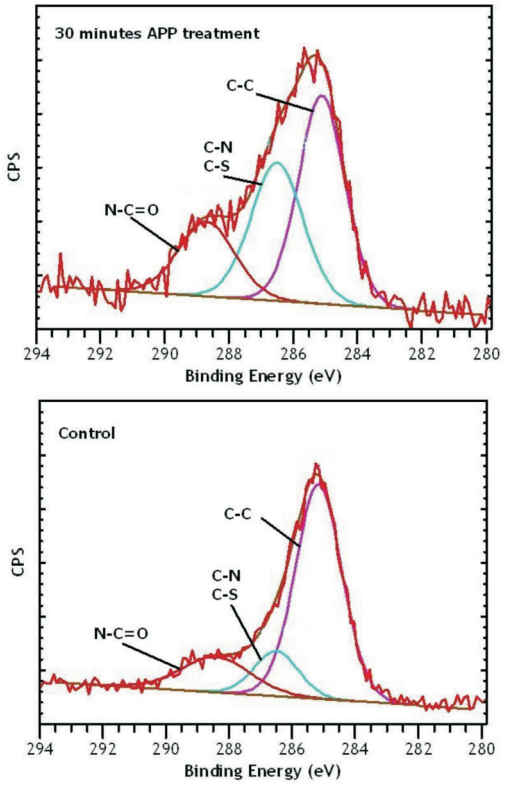

Fig. 2. XPS spectra of AVT after and before AP treatment.

The interpretation of the obtained results is quite difficult since AVT molecule has a very complicated structure. The main type of bonds in this molecule (that we should correlate with the spectra for the control sample) are: -NH_2, C-C(=O)-N, C-C, C-N-C, N-H, C-CH_3, C-S, one benzene with a OH , C-O, and a penta cycle with simple bonds between four Carbon atoms and a Nitrogen.

A rough analysis indicates that atmospheric plasma produces modification of Vasotocin, small changes after 10 and 20 minutes, and more profound after 30 minutes of exposure. XPS analysis indicated a strong reduction of S 2p peak possible indicating the break of disulfide bond in AVT molecules. The peaks corresponding to N-C=O and C-N from C 1s spectra exhibited a decrease after plasma irradiation (Figure 2), confirmed by N 1s spectra for the treated and control samples. The bonds involving oxygen in AVT samples also modified, -OH and O=C peaks decreasing.

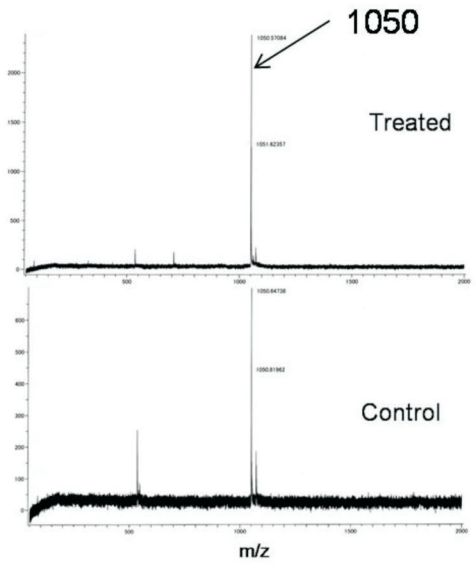

Fig. 3. TOF-MS spectra of AVT treated and control.

Treated Vasotocin kept its biological function after atmospheric plasma treatment and the mass was also conserved as TOF-MS measurements indicated.

3.2. Low pressure plasma treatment

In the other set of experiments, microwave excited surface wave plasma was produced. Samples were prepared on Silicon substrates and exposed for the same time intervals. The evaluated results indicated a stronger effect than for the atmospheric pressure plasma, which was expected due to higher particles energies and maybe also presence of a larger range of radiations including UV.

Same tests were employed as for the other samples. Sulphur S 1s peak caused by the presence of disulfide bond was not present after sample irradiation even for 10 minutes. The intensity of Nitrogen 1s spectrum strongly decreased, indicating the breaking of C – N bondings, fact confirmed by the fitting of C 1s peak. Disulfide bond causes a peak around 165 eV for the control Vasotocin sample but it doesn't exist anymore for the treated vasotocin. Results indicate that the structure of the molecule was modified by rearranging of its three dimensional structure. Plasma processing did not fragmentize

the peptides as TOF-MS spectra of treated and control samples show (Figure 3). The conformational change induced by plasma processing is also proved evaluating the biological function of Arginine Vasotocin molecules after treatment. The procedure consists in measuring the time evolution of the water volume flow through a small part of frog abdominal skin. The results are shown in Figure 4, in blue for the control sample, and in red for the 30 minutes processing. As measurements indicate, the exposed molecules don't contribute anymore to osmoregulation and we assume that this is due to the conformational change caused by plasma treatment.

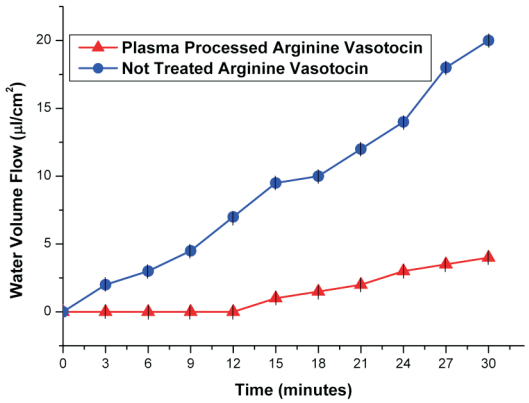

Fig. 4. Evaluation of AVT biological function.

4. Conclusions

Our study proved that low temperature plasmas are able to produce modifications of biopolymers, such as peptides. Plasma processing determined the rearrangement of the three dimensional structure without altering molecular mass, which means without fragmentize the molecule. Moreover, the changes induced by the low-pressure plasma exposure caused the lost of biological function of osmoregulation. The new conformation was not able to bind the receptor and produce the opening of the water channels in frog belly. Further investigations are necessary to improve the treatments and also elucidate the produced modifications and the interaction mechanisms.

ACKNOWLEDGMENTS

This work has been partly supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS).

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